11/22/83 H. Warpeha

Mini-Review of Literature on TSNA in Cigarette Smoke
— With emphasis on formation & prevention.

Schmeltz et al, J. ag. Fd. Chem. 27(3), 602 (1979)
Fate of nicotine in a burning cigt.

Using radiolabled nicotine it was determined that 41% of nicotine transfers into both MS and SS, more into SS than MS. 3-11% is converted to other volatile bases, more in SS than MS; in particulate phase.

most products from pyrolysis of nicot. during smoking are higher in SS than MS.

<0.1% of nicot. in filler contributes to smoke toxicity — this <0.1% accounts for dibenzacridine, TSNA, etc.

Hoffmann, 1973

Cigt. + nornicotine (8 x normal) —smoke→ NNN (50% higher than control)

Cigt. + nicotine (2 x normal) —smoke→ NNN (3 x control)

∴ Both nicotine and nor-nicot. can serve as precursors for NNN. However, in normal tobaccos nicotine : nornicotine ≅ 15 or 20 : 1

∴ nicotine is the most important precursor of NNN in cigt. smoke.

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Hecht, 1976 Transfer rate of NNN to MS smoke.
(^{14}C-NNN added to filler) US commercial NF blended cigt.

NNN in filler $\xrightarrow[11.3\%]{}$ NNN in smoke (MS) (110ng)

This was 46% of the total NNN found in MS (238ng)

∴ NICOTINE in filler $\longrightarrow$ NNN (128ng) in MS smoke is responsible for 54% of the NNN found in MS.

Adams et al, Cancer Letters, 17, 339-346 (1983) Transfer rate of NNK into MS smoke.

^{14}C-NNK transferred into MS smoke at the rate of 6.9-11.0%.

∴ 26-37% of NNK in MS smoke comes from NNK in filler.

^{14}C-nicotine added to tobacco column $\xrightarrow{\text{smoke}}$ determine NNK.
0.001% of ^{14}C-nicot. $\longrightarrow$ ^{14}C-NNK in MS

∴ 63-74% of NNK formed during smoking

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Mechanistics

Hoffmann, NCI #18, p177 (1970)

NO_2^- decomposes to NO, NO_2 and O_2 > 450°C. This must occur in the burning cone. The cone contains ~ 8% H_2 (a reducing atmosphere) NO_2 produced in the cone is either reduced to NO or acts as a scavanger for organic radicals. It takes 500 seconds for ½ the NO in smoke to oxidize to NO_2. NO will not nitrosate amines – requires $NO_2 \rightleftharpoons N_2O_4$ or N_2O_3

Mergens (1975)

believes the NA form from the following mechanism:

$NO \xrightarrow{O_2} NO_2$ (after coal)

NO_2 + 2° amines ⟶ NA

this may occur to some extent, and is probably the mechanism of formation in aged smoke, but is not likely to be the mechanism of formation during actual conditions.

Challis, British J. cancer 35, 693 (1977), Rapid formation of carcinog. NA in aqueous alkaline solution.

sealed flask containing 5ml 2°amine in 0.1M NaOH + nitrogen oxides at atmospheric pressure (N_2O_4 added in air; N_2O_3 added in O_2-free atmosphere)

① NA formation was proportional to $[NO_x]$.

② only the unprotonated amine was reactive

③ undissoc. in Ag Phase { $N_2O_4 \rightleftharpoons 2NO_2$ } dissociated { $N_2O_3 \rightleftharpoons NO + NO_2$ } in gasphase

④ NO_2 could abstract a H to give $R_2N^\bullet \xrightarrow{NO} R_2N-N=O$

⑤ metal ions required for radical formation.

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Source: https://www.industrydocuments.ucsf.edu/docs/jmkk0000

Hecht et al, 30th TCRC, 1976 — Studies on non-volatile NA in tobacco.

gives results of reaction of nicotine with nitrite in aqueous solution. Major products are cis- and trans-(3-pyridyl) acrylonitriles and N'-methylnicotinamide. The TSNA NNN and NNK are formed in low yields. NNA is also formed, but reacts further if drastic conditions are employed (such as 90°C and excess nitrite). Under the more drastic conditions the yield of NNN was 10%.

(see proposed mechanism on next page)

The mechanism shown here for aqueous solution can be considered to represent what may happen in smoking as well, unless a radical mechanism is predominant, or the reaction takes place in a lipid-like environment. Even in the latter case, the same mechanism may exist, since the free base is volatile, and could react with NO_2 or N_2O_4.

Some of the other products (top of page) may be potential indicators of the reaction taking place by this kind of mechanism.

Mergens et al IARC #19 (1979) pp 199-212.
This paper is covered later under inhibitors. It was stated that addition of α-tocopherol to cigts. gave 50% redn. in NDMA. Represents application to a system having lipophilic character. The smoke aerosol = the "lipid".

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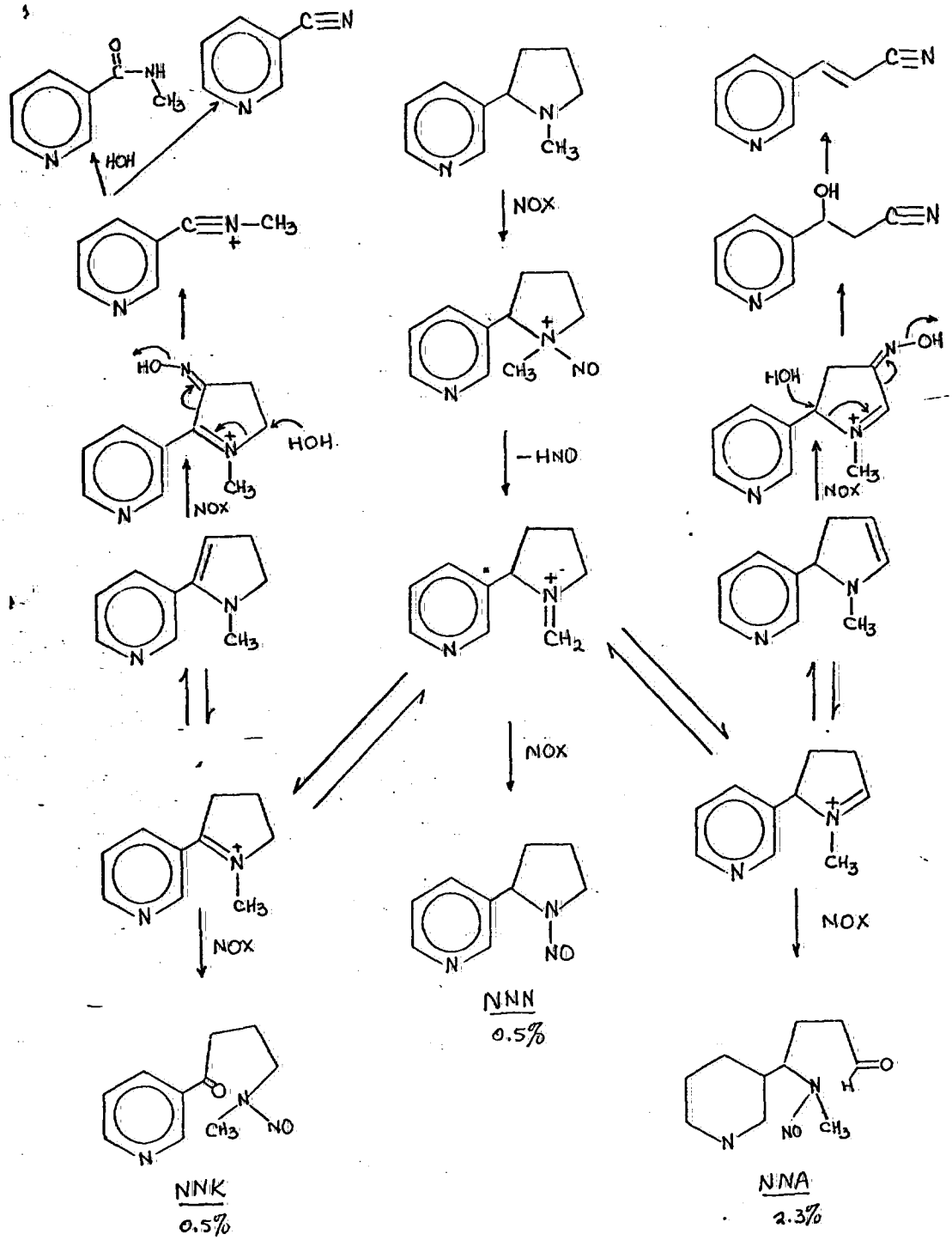
Source: https://www.industrydocuments.ucsf.edu/docs/jmkk0000

PROPOSED MECHANISM TO EXPLAIN PRODUCTS OBSERVED WHEN NICOTINE IS REACTED WITH $NaNO_2$, 20°/17Hr, pH 2.0 to 7.0 (OPTIMUM = 4.5)



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Source: https://www.industrydocuments.ucsf.edu/docs/jmkk0000

Considerations of MS/SS distribution of TSNA

Hoffmann Paper O-19, 8th IARC, 1983

MS & SS smoke parameters:

	MS	SS
Peak Temp. of Formation, °C	~900	~600
Particle size, nm	0.1–1.0	0.01–0.1
Smoke Dilution (vol %)		
CO	3–5	~1
CO_2	8–11	~2
O_2	12–16	16–20
H_2	3–15	~0.5

Tar is 30–80% higher in SS than in MS
amine yields are 10–30X higher in SS than MS
NH_3 " " 40–130X " " " " "
NO " " 4–10X " " " " "

also, the high dilution in SS leads to rapid opidation of NO to NO_2. SS VNA are greatly affected by varying the flow rate through the chamber, as anticipated, due to opid. to NO_2 and subsequent formation of VNA.

Generally, there is not much data in the literature in which reliable values have been obtained for directly comparing SS and MS TSNA.

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Source: https://www.industrydocuments.ucsf.edu/docs/jmkk0000

(presented)
One paper, however, has been published w/ reasonable data on SS & MS:

Brunnemann, Masaryk & Hoffmann, TCRC, 1983
Role of Tobacco Stems in Formation of NA in Tobacco and Cigt. MS and SS Smoke

9 experimental cigts. were prepared w/o filters, from 70% lamina and 30% various stems. Separate smokings were used to measure MS & SS smoke for TSNA.

Conclusions: MS smoke
1. TSNA in MS were strongly correlated w/ nitrate in filler
2. SS smoke delivered high amounts of NDMA
3. Yields of NDMA and NNN in SS were correlated w/ Nitrate in blend.
4. The nitrate content of stems had an important role in formation of TSNA during processing, as well as by pyrosynthesis.
5. Addition of 0.1% ascorbic acid to stems did not inhibit formation of VNA or TSNA in smoke.

Filler Data (ug/g)

Cigt#	Stems (Treatmt)	% NO_3	NNN	NAT	NAB	NNK
1	Bright	1.03	1.86	0.81	0.06	1.23
2	Bright (LN)	0.76	1.17	0.22	ND	0.56
3	Bright (Brazil)	0.91	1.19	0.67	0.03	0.75
4	Bright (Georgia)	1.00	1.16	0.24	ND	0.54
5	Bright (NC)	0.88	1.02	0.40	0.03	0.54
6	Burley (LN)	1.35	2.60	0.39	0.06	0.79
7	Burley (HN)	2.65	6.35	0.67	0.08	1.48
8	Br (LN) (3% K_2CO_3)	0.79	1.24	0.42	ND	0.69
9	Br (LN) (0.1% Ascor.)	0.68	1.04	0.42	ND	0.48

(above data normalized to NNN = 1.00)

1	1.00	0.44	0032	0.66
2	1.00	0.19	ND	0.48
3	1.00	0.56	0.025	0.63
4	1.00	0.21	ND	0.47
5	1.00	0.39	0.029	0.53
6	1.00	0.15	0.023	0.31
7	1.00	0.11	0.013	0.23
8	1.00	0.34	ND	0.56
9	1.00	0.40	ND	0.46

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Source: https://www.industrydocuments.ucsf.edu/docs/jmkk0000

TSNA in MS smoke, ng/cigt.

Cigt.	TPM	NNN	NAT	NAB	NNK
1	16.5	156	120	14	87
2	16.9	92	84	11	54
3	16.4	98	85	10	50
4	16.5	81	82	10	46
5	15.8	90	89	10	47
6	16.3	230	130	16	160
7	16.9	390	167	18	240
8	17.3	125	102	15	84
9	16.9	96	85	11	61
	(above data normalized @ NNN = 1.00)				
1		1.00	0.80	0.09	0.56
2		1.00	0.91	0.12	0.59
3		1.00	0.87	0.10	0.51
4		1.00	1.01	0.12	0.57
5		1.00	0.99	0.11	0.52
6		1.00	0.57	0.07	0.70
7		1.00	0.43	0.05	0.62
8		1.00	0.82	0.12	0.67
9		1.00	0.89	0.11	0.64
average:		1.00	0.81	0.10	0.60

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Source: https://www.industrydocuments.ucsf.edu/docs/jmkk0000

TSNA in SS Smoke, ng/cigt.

Cigt #	NNN	NAT	NAB	NNK
1	141	61	35	217
2	110	72	20	201
3	123	75	15	233
4	119	84	22	278
5	137	91	22	293
6	114	94	22	228
7	390	220	40	540
8	131	84	16	215
9	185	97	20	250
	(above data normalized to NNN = 1.00)			
1	1.00	0.43	0.25	1.54
2	1.00	0.65	0.18	1.91
3	1.00	0.61	0.12	1.89
4	1.00	0.71	0.18	2.34
5	1.00	0.66	0.16	2.14
6	1.00	0.82	0.19	2.00
7	1.00	0.56	0.10	1.38
8	1.00	0.64	0.12	1.64
9	1.00	0.52	0.11	1.35
average:	1.00	0.62	0.16	1.77

Summary:

	NNN	NAT	NAB	NNK
Filler	1.00	0.1-0.6	ND-0.03	0.2-0.6
MS	1.00	0.4-1.0	0.05-0.12	0.5-0.6
SS	1.00	0.4-0.8	0.1-0.25	1.4-2.3

Ratios in MS are similar to those in Filler.
In SS, NNK is formed at much higher rates relative to NNN.

This observation was not noted in the paper.
The MS & SS were not collected in the same experiment.
(Could be due to selectivity during formation of MS & SS due to area of the cone in which the reaction occurs)

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Source: https://www.industrydocuments.ucsf.edu/docs/jmkk0000

Data from Europe tend to confirm the results from AHF:

2R1 – average of 10 smokings

ng/cigt

	NNN	NAT	NAB	NNK
MS	150	193	39	145
SS	120	130	54	223

(normalized to NNN = 1.00)

MS	1.00	1.29	0.26	0.97
SS	1.00	1.08	0.45	1.86

Note that NAB also doubles and NAT is lower. The same holds true for AHF data on p. 9

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Source: https://www.industrydocuments.ucsf.edu/docs/jmkk0000

Reduction of TSNA in smoke

Hoffmann et al N-NA in Tob Prod.
ACS Symposium Series 174 p 268 (1981)

Reduction of TSNA in Tob Smoke

(Research goal at AHF) Devel. of concepts for Redn. of TSNA in smoke.

"further redn. of nic. below 0.6–1 mg/cigt. leads to more intense smoke inhal. in order to reach his/her "satisfaction level" for nic. etc." "

Variation of TSNA w/ puff volume – see Fig 10. – beyond levels of normal tar cigts. for low tar cigts.

Selective redn w/ filters vs NF – "Black Tobacco Cigts"

	CA filter (% Redn)	Cellulose (% Redn)
TPM	40	50
NIC	42	52
NNN	69	77
NNK	56	72
NAT	72	75
TSNA	69	76

Selective redn of TSNA by perforated CA filter tips w/ high air dilu. – Smoker compensates by smoking more intensely, but TSNA doesn't increase to level observed w/ unperforated cigt.

Later papers by AHF included statements that the example of French black tobacco is the only one where any such effect is demonstrated, and it is explained by a higher pH of the smoke.

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Source: https://www.industrydocuments.ucsf.edu/docs/jmkk0000

TABLE VIII

SELECTIVE FILTRATION OF TOBACCO SPECIFIC N-NITROSAMINES
EXPERIMENTAL CIGARETTES WITH BLACK TOBACCO (85mm)

Parameter	Cigarette 1 Nonfilter	Cigarette 2 20mm - Filter (Cellulose Acetate)		Cigarette 3 20mm - Filter (Cellulose)	
	Value	Value	% Reduction	Value	% Reduction
"Tar"-FTC (mg)	18.2	10.9	40	8.1	50
Nicotine (mg)	2.1	1.2	42	1.0	52
NNN, ng/cig	3,200	1,000	69	730	77
NNK, ng/cig	430	190	56	120	72
NAT, ng/cig	640	120	72	160	75
Total TSNA, ng/cig	4,270	1,310	69	1,010	76

[a]Number in parentheses represents the number of the last puff taken.
Abbreviations: see footnote 2 of Table V.

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Source: https://www.industrydocuments.ucsf.edu/docs/jfnk0000

IARC #41 (1984)

RESULTS

Analytical data for cigarette smoke

Fig. 2 depicts a representative chromatogram of the TSNA in cigarette smoke. Although values for TSNA in cigarette smoke are in good agreement with those obtained earlier by HPLC-TEA, the GC method is preferred because it is faster, less cumbersome and affords a clear separation of NAB from NAT. The GC-TEA method showed that cigarette smoke contains up to 15 ng of NAB and 650 ng of NAT, and it is now being applied to test the efficiency of cigarette filter-tips with respect to selective TSNA removal. It appears the perforation of the filter tip (to dilute the smoke) offers potential for such selective reduction of TSNA in the mainstream smoke.

Table 1 summarizes some of the data obtained from smoke analyses of selected commercial cigarettes with and without filter tips and shows the effectiveness of the filters of cigarettes D and E. At present, we are exploring the physical and chemical conditions which provide optimal selective removal of both volatile and tobacco-specific nitrosamines. In that respect, the degree of smoke dilution obtained by filter perforation and the nature of the plasticizers in the filter tip appear to be dominant factors.

Table 1. Reduction of tobacco-specific *N*-nitrosamines in cigarette smoke by filtration[a]

Cigarette		Column smoked/length of cigarette (mm)	'Tar' (ng)	Nicotine (mg)	CO (mg)	NNN (ng)	NNK (ng)	NAT (ng)	TSNA (ng)
A	F	50/85	13.0	1.10	15.6	458	360	570	1390
			-40%	-40%	+4%	-44%	-50%	-47%	-47%
	NF	50/65	21.8	1.82	15.0	816	720	1080	2620
B	F	50/85	14.2	1.31	18.5	324	252	390	966
			-37%	-29%	+9%	-39%	-48%	-47%	-45%
	NF	50/65	22.7	1.84	16.9	533	481	735	1750
C	F	50/85	13.6	1.15	13.7	505	399	670	1570
			-40%	-30%	+13%	-38%	-45%	-39%	-40%
	NF	50/65	22.5	1.66	12.1	813	727	1080	2620
D	F	50/85	12.9	1.05	15.3	342	213	352	907
			-45%	-34%	+3%	-69%	-73%	-70%	-71%
	NF	50/65	23.3	1.60	14.9	1110	803	1260	3170
E[b]	F	50/100	13.4	1.08	12.0	513	249	593	1360
			-39%	-34%	-20%	-71%	-71%	-69%	-70%
	NF	50/73	21.8	1.63	15.0	1760	868	1930	4560

[a] The percentages are the changes in yields on comparing filtered (F) and non-filtered (NF) smoke.

[b] Cigarette E had a perforated filter-tip.

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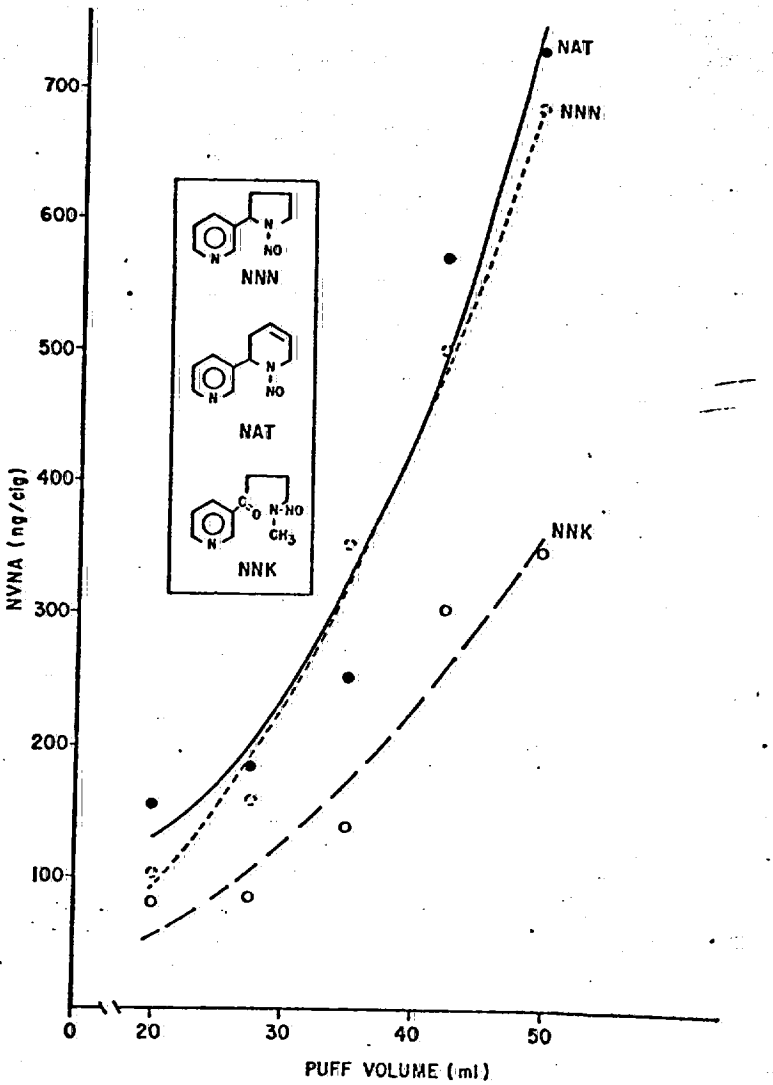

Figure 10. Tobacco specific nitrosamines as a function of puff volume (other smoking conditions were kept constant: puff duration, 2 s; puff frequency, once a minute; and butt length, 23 mm).

Source: https://www.industrydocuments.ucsf.edu/docs/jmkf0000

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Mergens et al IARC #19 (1978) pp 199-212

alcoholic solns. of α-tocophorol applied to 2R1 filler @ 30mg / 1.1g, and 1.1g cigarettes, 85mm NF, made.

Smoke collected in ECT / -80°C

NDMA isol. by steam dist. from 2N NaOH, extr. w/ CH_2Cl_2, chrom. on SiO_2 + Al_2O_3. GC / Hall EC detector.

an average reduction in NDMA of 50% was observed

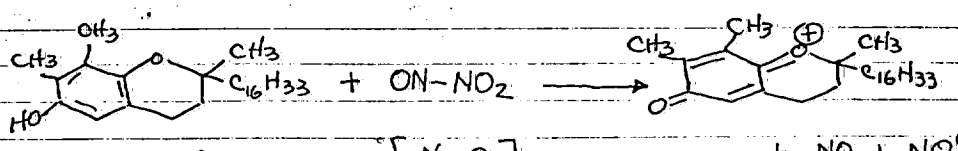
mechanism: DMA + N_2O_3 ⟶ NDMA (lipid phase)

α-Tocophorol + N_2O_3 ⟶ Oxidized α-tocophorol + NO + $NO_2^{\ominus}$
(lipid soluble)

"lipid phase" is the smoke aerosol

This is a possibility in the case of TSNA as well.

The above result is in serious doubt because of the use of an ECT, which invites artifact formation.



α-tocophorol [$\equiv N_2O_3$] + NO + $NO_2^{\ominus}$

neither NO or $NO_2^{\ominus}$ can nitrosate in absence of H_3O^+

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Source: https://www.industrydocuments.ucsf.edu/docs/jmkk0000